NTP
National Toxicology Program

Research Concept on
Xylenes

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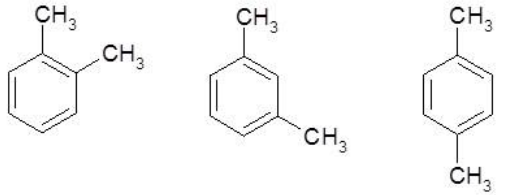
Xylenes – Overview and Nomination History

- Alkylated monoaromatic hydrocarbons produced in high volume and found in all environmental media
- Nominated for NTP testing on multiple occasions by different organizations for a variety of endpoints
- NTP has conducted prechronic and chronic toxicity and carcinogenicity studies via oral gavage
- Numerous studies; however, significant data gaps remain
- Agency for Toxic Substances and Disease Registry (ATSDR) and Integrated Risk Information System (IRIS) Assessments



o-xylene *m*-xylene *p*-xylene

Xylenes- Production, Use and Human Exposure Potential

- Produced commercially from petroleum
  - US Annual Production: >8 billion pounds
  - Approximate isomeric ratio ~40-44% *m*-xylene, 20% *o*-xylene, 20% *p*-xylene, 15-20% ethylbenzene; minor contaminants (toluene)
- Uses
  - ~70%: production of ethylbenzene and individual isomers
  - ~30%: Solvents, paints and coatings
- Benzene, Toluene, Ethylbenzene and Xylene (BTEX)
  - Most soluble of the major gasoline compounds – common indicators of groundwater contamination
- Human exposure: primary route is inhalation
  - Releases into air > water by 2-3 orders of magnitude
  - Industrial emissions, automobile exhaust, solvents
  - Oral exposure: contamination of soil and water; presence in some foods

Disposition and Primary Targets of Toxicity

- Disposition:
  - Rapidly absorbed and well distributed following inhalation or oral exposure
  - Metabolized primarily by liver
  - Eliminated primarily in urine as glycine conjugate or via expired air
- Primary targets of toxicity in humans and animals: respiratory tract and central nervous system
- Cancer
  - Human data inadequate
  - No inhalation carcinogenicity data available in animals
  - Negative for carcinogenicity following oral exposure

Exposure Route Consideration for Carcinogenicity of BTEX and related compounds

Test Agent	NTP Report	Route	Male Mice	Female Mice	Male Rats	Female Rats
Benzene[a]	289	Gavage	CE	CE	CE	CE
Toluene	371	Inhalation	NE	NE	NE	NE
Ethylbenzene	466	Inhalation	SE	SE	CE	SE
Xylenes[b]	327	Gavage	NE	NE	NE	NE
Styrene[a]	185	Gavage	EE	NE	NE	NE
Cumene	542	Inhalation	CE	CE	CE	SE

[a]Carcinogenic via inhalation (non-NTP studies)

[a]Test agent contained 14% ethylbenzene

Clear Evidence; Some Evidence; Equivocal Evidence; No Evidence

Exposure Route Consideration for Carcinogenicity of BTEX and related compounds

- Structurally similar compounds are positive for carcinogenicity via inhalation exposure
  - Target sites included the respiratory tract (lung, nose)
- Recent NTP Report on Carcinogens activity for styrene (listed) and cumene (recommended for listing)
  - *Reasonably anticipated to be a human carcinogen*
- Evidence of carcinogenicity for a particular test agent may differ following inhalation vs. oral exposure
- Xylenes have not been tested for carcinogenicity via inhalation
- Are xylenes similar to cumene/ethylbenzene or toluene?

Other Database Limitations

- Reproductive toxicity
  - No effects on reproductive parameters in a one generation inhalation reproductive toxicity study
  - Conflicting evidence on effects in male reproductive tissue weights and sperm parameters
- Developmental toxicity
  - Fetal effects: multiple inhalation studies – skeletal abnormalities
    - Associated with maternal toxicity in most studies
    - Major design differences among studies; limitations in some studies: non-guideline; lack of litter based statistics; lack of reporting of maternal toxicity
- Neurotoxicity
  - CNS effects in adult humans and animals well characterized
  - Limited evidence of developmental neurobehavioral effects
    - Similar concentrations as adults but lack of dose-response data

Rationale

- Further studies to characterize the toxicity and carcinogenicity of xylenes following inhalation exposure are warranted
  - Magnitude of production
  - Use patterns in occupational and consumer settings
  - Observed effects in the respiratory tract
  - Limitations in the available data sets for toxicity and carcinogenicity in humans and animals

Key Issues – Test Agent

- Test Agent – purity and presence of ethylbenzene
  - Options
    - Commercial preparation of mixed xylenes (~15-20% ethylbenzene)
      - Ethylbenzene
    - High Purity mixture of the three xylene isomers
    - Individual isomers
  - Propose to test a high purity mixture

Key Issues – Route of Exposure and Endpoints

- Database deficiencies
  - Chronic inhalation toxicity/carcinogenicity
  - Reproductive, developmental and neurotoxicity in developing animals
- Generation of data on multiple endpoints under the same exposure scenario
  - Efficiencies in chemistry, exposure system design and generation/monitoring, and animal husbandry, etc
  - Ability to make direct dose-response comparisons
- Logistical challenges in conducting developmental exposures via inhalation
  - Exposure and husbandry of dams and offspring during the lactation phase
  - Time constraints resulting from performing exposures, husbandry of dams/offspring and functional assessments within the same day

Specific Aims

1. Obtain a high purity mixture of *o*-, *m*-, and *p*-xylene free of ethylbenzene for use as a test agent in the conduct of toxicity and carcinogenicity studies (aims 2 and 3)
2. Evaluate the subchronic toxicity and chronic toxicity and carcinogenicity of xylenes following whole body inhalation exposure
3. Evaluate the developmental, reproductive and neurotoxicity of xylenes following whole body inhalation exposure

Test Agent (Specific Aim 1)

- Several commercially available preparations of mixed xylenes will be analyzed to determine representative ratios of individual xylene isomers
- Individual isomers at a high purity will be procured and blended to an isomeric ratio representative of commercially available preparations of mixed xylenes
- Resulting test agent will be a high purity mixture of *o*-, *m*-, and *p*-xylene isomers

Toxicity and Carcinogenicity Studies (Specific Aims 2 and 3)

- Conduct subchronic and chronic whole body inhalation toxicity and carcinogenicity studies of xylenes
- Include exposure of additional animals to evaluate:
  - Reproductive and developmental toxicity endpoints
  - Neurotoxicity endpoints (following developmental exposure)
  - Selection of endpoints will be tailored to provide robust data in the context of the logistical challenges of exposure of developing animals via inhalation
- Short term studies on individual isomers to evaluate potential differences in toxicities

Significance and Expected Outcomes

- The production, use and human exposure potential of xylenes are significant
- Human and animal datasets have significant weaknesses
- Proposed research program will
  - Generate data to evaluate whether potential toxicity and carcinogenic effects can be assigned to xylenes
  - Address significant data gaps
  - Provide basis for dose-response comparisons across multiple endpoints to examine relative sensitivities
  - Improve confidence in the risk assessments on xylenes

Review Questions

1. Comment on the merit of the proposed project relative to the mission and goals of the NTP. *The NTP's stated goals are to: Provide information on potentially hazardous substances to all stakeholders; Develop and validate improved testing methods; Strengthen the science base in toxicology; Coordinate toxicology testing programs across DHHS (http://ntp.niehs.nih.gov/go/about).*

2. Comment on the clarity and validity of the rationale for the proposed project. Has the scope of the problem been adequately defined? Are the relevant knowledge gaps identified and clearly articulated?

3. Comment on the strategy and approach proposed to meet the stated objectives of the project. Are specific aims reasonable and clearly articulated? Is the scope of work proposed appropriate relative to the public health importance of the issue(s) under consideration? If not, what modifications do you recommend? Where steps to further refine the strategy and/or approach are proposed, are they appropriate?

4. There are challenges inherent to achieving the aims of any proposed project. Are the relevant challenges and/or key scientific issues identified and clearly articulated? Where approaches to overcome challenges are proposed, are they appropriate? Are you aware of other scientific issues that need to be considered?

5. Rate the overall significance and public health impact of this project as low, moderate, or high. Identify any elements of the proposed project that you feel are more important than others, and/or that have a higher likelihood of success at meeting pre-defined specific aims.

1. Provide any other comments you feel NTP staff should consider in developing this project.